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28 December 1997

TITLE OF INVENTION

PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND USE LASER TWEEZERS

APPLICANT(S) FOR DO/EO/US

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MONAJEMBASHI, Shamci

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☒ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

PCT Request; International Publication (Cover page); a postcard and a check in the amount of \$1,352.

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Günter Fuhr et al.
Serial No. : Unassigned Examiner: Unassigned
Filed : Herewith Group Art Unit: Unassigned
For : PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND
USE LASER TWEEZERS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to calculation of the filing fee, entry of this Preliminary Amendment is respectfully solicited.

IN THE CLAIMS:

Please cancel claims 1-27.

Please add claims 28-54 as follows:

-- 28. A procedure to measure or exert optically-induced forces on at least one particle in the focus of an optical cage with the following steps:

- a) the focus is positioned in a microelectrode arrangement with an electrical field that has a field gradient which forms a three-dimensional electrical capture area and the focus is at a distance from the capture area, and
- b) the amplitude of the electrical field, the light power of the light beam forming the optical cage, and/or the distance of the capture area from the focus are varied to detect at which of said varied field properties the particle is moved from the focus to the capture area or vice versa, or to at least to temporarily move the particle into the capture area.--

--29. The process according to claim 28 in which a particle is placed in the focus or capture area to measure optically-induced forces, and the optically-induced forces are measured from the amplitude of the electrical field and the distance of the capture area from the focus when the particle moves from the focus to the capture area or vice versa.--

--30. The process according to claim 29 in which the optically-induced forces are repeatedly measured for all relevant directions in space corresponding to mutual alignment of the positions of the focus to the capture area.--

--31. The process according to claim 29 in which the optical cage is calibrated by determining the relationship between the light power to generate the optical cage and the forces induced on a particle in the optical cage.--

--32. The process according to claim 1 in which the distance between the focus and capture area is at least one-tenth of the particle diameter.--

--33. The process according to claim 1 in which the capture area is a capture point that is in the beam field of the optical cage so that the particle moves back and forth between the capture point and focus when the amplitude of the electrode signals or light power is lowered or increased, and the associated value of the amplitude is used to measure the optically-induced forces.--

--34. The process according to claim 1 in which numerous particles are sequentially injected into the capture area that are positioned in set positions with the optical cage in the capture area relative to possibly existing particles in the capture area.--

--35. The process according to claim 1 in which the light beam of the optical cage is adjusted and/or the capture quality, symmetry or other calibration properties of the optical cage are measured.--

--36. The process according to claim 1 in which the particle is characterized based on the measured optically-induced forces.--

--37. A process to determine bonding forces between the microscopic particles, in which at least one particle is in the focus of an optical cage and at least one second particle is in the three-dimensional capture area of an microelectrode arrangement, whereby the first and second particles contact each other for a predetermined contact period, and then the amplitude of the electrical field, the light power and/or the distance of the capture area from the focus are varied until the first particle can be moved with the focus from the capture area and second particle, whereby the binding forces between the particles are determined from the amplitude of the electrical field and the light power when the particle moves.--

--38. The process according to one of claims 28 or 37 which the electrodes of the microelectrode arrangement are alternatingly supplied with signals phase-shifted 180° and/or with rotation-generating signals with a predetermined phase division.--

--39. The process according to one of claims 28 or 37 in which the capture area is separated by at least one field barrier from the optical cage.--

--40. The process according to one of claims 28 or 37 in which the particle movement is optically and/or electrically detected.--

--41. The process according to one of claims 28 or 37 in which the particles are synthetic or natural particles with a size below 200 μm .--

--42. The process according to one of claims 28 or 37 in which the particles are biological cells or their components.--

--43. The process according to one of claims 28 or 37 in which the transitional movement of the particle from the capture area to the focus or vice versa is used to adjust the optical cage.--

--44. A device to measure or exert optically-induced forces on at least one particle in the focus of an optical cage that comprises:

- a fluid microsystem with a microelectrode arrangement that is set up to form an electrical field with a three-dimensional electrical capture area,
- an illuminating device that is set up to form an optical cage in the microelectrode arrangement of the microsystem, and
- an monitoring and/or detection device to measure the movement of particles in the microelectrode arrangement.--

--45. The device according to claim 44 in which the microelectrode arrangement comprises flat electrodes that are in groups on two spaced substrates of which at least one is transparent.--

--46. The device according to claim 45 in which the thickness of the transparent substrate is less than 500 μm .--

--47. The device according to claim 45 in which the electrodes are attached to facing surfaces of the substrates, and the substrates are separated from each other by a spacer that forms a suspension area into which the focus of the optical cage can be coupled by the illumination device through one of the two substrates.--

--48. The device according to claim 47 in which the suspension area is part of a channel structure through which the particles are introduced by means of a flow of solution into the field of the microelectrode arrangement.--

--49. The device according to claims 44 in which the microelectrode arrangement comprises numerous electrodes that are set up to generate a multiple field with an electrical field distribution symmetrical in the x, y and/or z direction.--

--50. The device according to claim 44 in which the electrodes are coated with an insulating, dielectric layer or consist of metals that are essentially inert to the suspension liquid in the microsystem.--

--51. The device according to claim 50 in which the electrodes consist of platinum, titanium, tantalum or gold.--

--52. The device according to claim 44 in which the electrodes are constructed in three-dimensional shapes using methods from semiconductor technology, or are constructed using hybrid techniques.--

--53. Method of using a procedure according to claim 1 to calibrate a laser tweezer.--

--54. Method of using a procedure according to claim 1 or 10 to selectively stimulate biological cells.--

REMARKS

This Preliminary Amendment is filed to remove certain multiple dependencies and to otherwise place the claims in better condition for United States Patent Office practice.

Respectfully Submitted,



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Applicant or Patentee: Fuhr et al.

Serial or Patent No.: Filed or Issued:

For: PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND USE LASER TWEEZERS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

- ☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to
 act on behalf of the concern identified below:

NAME OF CONCERN EVOTEC BioSystems AG

ADDRESS OF CONCERN Schnackenburgallee 114, D-22525 Hamburg, Germany

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND USE LASER TWEEZERS by inventor(s) Gunter Fuhr, Thomas Schnelle, Torsten Müller, Hermine Hitzler, Karl-Otto Grealich and Shamci Monajem described in

- ☐ the specification filed herewith
☐ Application Serial No. , filed
☐ Patent No. , issued

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which could not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).
 Note: Separate verified statements are required from each named person, concern or organization having rights to the invention availing to their status as small entities. (37 CFR 1.27)

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☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

Baker Botts L.L.P

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed

NAME OF PERSON SIGNING Dr. Karsten HENCOTITLE OF PERSON OTHER THAN OWNER Chief Executive OfficerADDRESS OF PERSON SIGNING Schnackenburgallee 114, 22525 Hamburg, Germany

SIGNATURE _____

DATE

28.7.2000Hamburg

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PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND USE
LASER TWEEZERS

The invention concerns processes and devices to measure and calibrate optical fields traps, determine optically-induced force in all three dimensions that are exerted on micrometer-sized particles, and to use optical field traps.

Optical field traps, also called "optical tweezers", "laser tweezers" or "optical traps" have been used for approximately two decades in the fields of biotechnology, medicine and molecular biology as well and other technical fields to position and manipulate micrometer-sized and submicrometer-sized particles (G. Weber et al. in Int. Rev. Cytol., Vol. 131, 1992, p. 1; S.M. Block in Noninvasive Techniques in Cell Biology, Wiley-Liss., New York 1990, p. 375). A. Ashkin has primarily started the development of laser tweezers (A. Ashkin in Phys. Rev. Lett., Vol. 24, 1970, p. 156). The principal of capturing particles by optically-induced forces is based on the fact that, in addition to radiation pressure that continuously pushes particles away from the light source, gradient forces arise which cause particles to move into a focus or be firmly held in it or move with it. Absorption and reflection of the particles must be low while the difference of the refractive index relative to the environmental solution should be as large as possible.

In recent years, laser tweezers have primarily gained acceptance because particles that are larger than the wavelength (Mie particles) as well as particles that are smaller than the wavelength (Rayleigh particles) can be trapped when the light beam is even and highly focussed. This includes biological objects such as cells, or organelles and other cell components as well as large molecules

(such as DNA) and artificial microparticles (S.M. Block et al. in Nature, 1990, p. 348; E.M. Bonder et al. in J. Cell Biol., Vol. 11, 1990, p. 421).

W. Paul discovered electromagnetic field cages (W. Paul et al. in Forschungsberichte des Wirtschaftsministeriums Nordrhein-Westfalen, No. 415 and 450; W. Paul in Phys. Blätter, Vol. 46, 1990, p. 227). They are primarily used in elementary particle physics to trap and measure atomic particles under low gas pressures. In 1993, liquid-filled, three-dimensional microfield cages were presented for the first time using dielectrophoretic forces (T. Schnelle et al. in Biochim. Biophys. Acta, Vol. 1157, 1993, p. 127). If the conductivity of a suspension solution is higher than that of the average conductivity of the particle, the surface charges on the particle induced in the E field are arranged and polarized to make effective the forces acting on the particle (G. Fuhr et al. in Biochim. Biophys. Acta, Vol. 1201, 1994, p. 353). This principle can also be used to trap, position and move particles (T. Schnelle et al. in Biochim. Biophys. Acta, Vol. 1157, 1993, p. 127). Objects corresponding to the Mie and Rayleigh particles can also be held in these dielectric field cages. There have been numerous suggestions for combining both principles to increase the trapping force since the optical and electrical force fields scarcely interfere with each other (G. Fuhr et al. in Topics in Current Chemistry, Vol. 194, 1998, p. 84).

A previously unsolved problem is how to measure the actual optically-induced force in the optical field (laser trap) acting on a particle. There are several extremely time-consuming methods to do this requiring much equipment that can be summarized as follows:

Method I (K. Svoboda et al. in Ann. Rev. Biophys. Biomol. Struc., 1994, p. 247).

A calibrated flow acts on a particle trapped in the focus of a laser. The flow speed is sought at which or just before the particles are torn from the laser focus. A capture force can be calculated from this flow speed using Stokes' law. A disadvantage of this procedure is that it is difficult to repeat measurements using the same particle, and very complicated channel structures are required to make measurements in different spatial directions. A three-dimensional measurement of force (x, y, z) is not possible. This procedure is also limited to certain particle shapes (spheres, ellipsoids) with smooth surfaces.

Method II (C.P. Dennis in Faraday Discuss. Chem. Soc., Vol. 90, 1990, p. 209):

The average deflection of a particle in the laser focus is measured based on Brownian collisions. In principle, all three dimensions can be measured; however, the particle motion must be measured very precisely with submicrometer precision which cannot be done for most Mie particles since they are too large for any notable deflection. In addition, the measurements become increasingly difficult and imprecise as laser power increases, and one can only work within a very narrow laser focal range.

Method III (C.P. Dennis et al. in Applied Optics, 1993 p. 1629):

The investigation concerns an attempt to tear away a particle that is affixed to a substrate in a specific manner using a laser tweezer. The scattering of an evanescent surface wave with total reflection is used to determine the motion of the object in the z direction (perpendicular to the surface) with a precision in the nm

range. This procedure also cannot be used in all three dimensions, and it is slow and requires a large amount of calibration and equipment. In addition, the optical radiation field close to the surface does not correspond to conditions in a free solution.

In general, there is no fast and easy-to-the use method to measure force that can be carried out in the later-to-be-used particle's environmental medium with the particle itself under the same conditions.

Another previously unsolved problem in handling microscopic particles is the measurement of intermolecular attractive forces (adhesion forces) that act between the particles. Both synthetic and natural particles based on biological materials (in particular, biological cells or cell components) tend to adhere to each other when they contact. Depending on the type of adhesion, adhesion phenomena can be divided into non-specific and specific adhesion or, depending on the type of bonds in the adhesion, it can be divided into adhesion with chemical bonds or adhesion with van der Waals bonds. Measuring the specificity, the bond type and hence the bond force of adhesion is between microscopic particles is of great interest to the fields of biology, medicine and pharmacology. There is therefore a need for quantifiable, repeatable, and non-destructive procedures to measure adhesion phenomena of individual particles, e.g. to measure the specific bonds of macromolecules (which also includes microscopic particles in this context) to biological cells to investigate the immune response or to recognize infection or other cellular diseases. Another example is the interaction between microscopic particles where specific receptor-ligand systems are active on the particle surface.

There does not exist any procedure for setting specific adhesion phenomena between individual suspended microscopic

particles, or for determining the occurring bond forces.

The article by K. Morishima et al. in Proc. Of IEEE, 1997, p. 155, discloses the combined use of optically-induced forces and electrical field forces to manipulate bacteria. In a microscopically-observed microsystem of a cluster of bacteria, a specific bacteria is trapped and fixed with a laser tweezer while the other bacteria are moved away from the individual, fixed bacteria under the effect of the electrical field. This procedure described by K. Morishima et al. is limited in its application to the described separation of individual particles from a group of many particles. The process solely concerns turning electrical fields on or off to separate particles. An investigation of the interaction between particles is not possible. In addition, individual particles cannot be moved to an existing group particles or an aggregate of particles to attain specific interactions.

The invention is based on the object of presenting improved procedures to handle particles with laser tweezers that can in particular be used to measure optically-induced forces or bonding forces, and to exert a specific force. In particular, a process according to the invention is to be capable to measure forces faster with better reproducibility and precision. The forces that act on microscopic particles are to be measured via an electric signal in quickly repeatable and is automated manner, wherein the force is to be measured with a precision in the pN range and below in any dimensional direction. Another object of the invention is to provide devices for implementing the procedure.

The above objects are solved by procedures and devices with the features according to claims 1 and 17. Advantageous embodiments and uses of the invention are defined in the dependent claims.

In particular, the invention provides capturing at least one microscopic particle in the focus of an optical cage in a microelectrode arrangement and positioning it in reference to an electrical capture area (or capture point) that is formed by electrical field gradients in the microelectrode arrangement. The focus of the optical cage with the particle is initially positioned at a distance from the capture area, i.e., at a distance from the minimum electrical field level of the capture area that represents an electrical field cage. Then the field forces in the optical cage and/or in the electrical capture area and/or the distance between the optical cage and electrical capture area are varied until the particle moves from the focus to the capture area or vice versa. Depending on the application, the given field properties when the particle starts to move are used to measure forces according to the invention, or the transitional movement itself is used to exert specific forces.

In the procedure according to the invention, forces are generated that act on a particle or particles whose fields possess a minimum level in a microelectrode arrangement with a three-dimensional electrical field that contains electrical field gradients forming the capture area. The minimum level of the electrical field forces corresponds to the capture area. The minimum level of optically-induced forces is the focus of the optical cage. Between both minimum levels is a field barrier that depends on the amplitude of the effective electrical fields, the light power of the optical cage, and the distance between the minimum levels. Depending on the setting of these quantities, the particle or particles can be positioned within the microelectrode arrangement in a predetermined manner or (if there are numerous particles) be separated from each other.

According to one important aspect of the invention, the optically-induced forces acting on a particle in the focus of an optical cage are measured. In this embodiment, only one particle is in the focus or temporarily in the capture area of the microelectrode arrangement. The field properties, i.e., the amplitude of the electrical field, the light power and/or the distance between the minimum levels is varied until the particle moves between the minimum levels, i.e., between the focus and the capture area or vice versa. It was surprisingly determined that the transitional movement can be determined very precisely in a reproducible manner. The optically-induced forces in the optical cage can be measured from the amplitude of the electrical fields in the microelectrode arrangement required to trigger the transitional movement.

According to a second important aspect of the invention, the bonding forces between several particles (e.g. to particles) are measured. In this embodiment, one particle is in the focus of the optical cage, and one particle is in the electrical capture area. The properties of the fields in the microelectrode arrangement can be varied to determine those field properties that make the particle in the focus move toward the particle in the capture area or vice versa. This principle can be correspondingly realized with groups of particles in the focus or the capture area.

The adhesive bonding force that acts between the particles can be measured from the electrical field force and (when the measurement of bonding force is combined with the measurement of optically-induced force according to the first embodiment) the optically-induced force by observing the transitional movement or tearing-away movement between the particles. A particular advantage of the invention is that the processes can be observed and evaluated in reference to specific materials, particles, and bonding types.

According to a third important aspect of the invention, microscopic particles in a microelectrode arrangement with the cited (at least) two minimum field levels with relative, set field force can be at least temporarily positioned or joined into groups or aggregates, or the groups or aggregates can be divided into parts. The manipulation of the aggregates according to the invention is preferably combined with the above-cited aspects of the invention; it can also be used independently within predetermined (if unknown) electrical and/or optical field strengths.

The subject to the invention is also a microsystem that is adapted to create an optoelectric double cage that is generated by the simultaneous creation of at least two minimum field levels of an electrical capture area and an optical cage. A fluid microsystem possesses a microelectrode arrangement to generate the electrical capture area and a transparent structure for creating the optical cage within the microelectrode arrangement. The microsystem is preferably a fluid microsystem that can be open on one side facing the light source to generate the optical cage.

It is to be noted that the techniques used to according to the invention for creating the microsystem, generating the electrical field strengths, and generating the optical cage are known as such so that details will not be discussed in the following description.

In the following, general explanations of basic principles of the invention will first be provided.

In the laser trapping process, a particle can be held in a local equilibrium that is formed by an optical trap or an optical cage in the focus of at least one laser beam. In a high-frequency microfield cage, a particle can be held in a local equilibrium that is formed by an electrical capture area of the respective field distribution. The capture area

can be formed by a point, a line, or an area depending upon the field distribution. In the following description, in a non-restrictive manner the capture area will be referred to as a point; the invention can, however be correspondingly implemented using any shape of capture area. According to the above-cited first aspect, the invention is especially based on measuring the optically-induced forces in the optical trap (optical cage) consisting of the electrical force exerted on the particle when it changes between the states of equilibrium, i.e., from the focus to the capture point or vice versa. The object is hence especially solved by means of laser trapping in an electrical high-frequency micro fieldcage whose field strength and electrical field distribution are known, and that is adapted to inject the laser light required to form the optical cage. By shifting the particle trapped in the laser trap (focus) from the capture point of the field cage at low electrical trap power into a specific position at a distance from the capture point, the threshold can be precisely determined at which the electrical field returns the particle from the optical focus to the capture point or vice versa by subsequently increasing the amplitude of the electrical control signals of the field cage.

The laser light required to form the optical cage is injected by means of various design features of the microfield cage. Among these are in particular attaching at least one partial group of electrodes of the microfield cage to a substrate that is transparent and thin enough for a laser light source to be brought close enough to the microfield cage so that the focus forms in it. In practical designs of the laser tweezers, the laser light source comprises an coupling lens with a very high numerical aperture. The focal length is normally in the range of a few hundred micrometers. The preferred thickness of the transparent substrate is therefore less than the focal length of the laser light source.

Depending on the application, the invention allows measurement of qualitative and/or quantitative parameters of the optically-induced forces exerted on a particle. The optically induced forces can be quantitatively measured from just a few quantities that e.g. comprise the location of the focus and the capture point, the field distribution between the electrodes, the electrical properties of the particle and its environmental solution as well as the shape, phase angle, frequency and amplitude of all the electrode signals. All of these quantities can be determined independent of the actual measurement, or beforehand using purely electrical means, or by means of a single numeric simulation of the field distribution in the high-frequency cage. The optically-induced forces that act on the particle can be measured from the amplitude of the electrical control signals of the electrical field cage when the particle changes equilibrium (transitional movement). By systematically moving a particle whose passive electrical properties are known in the x and/or y and/or z direction, the forces from the focussed laser beam that act on a particle of this size can be quantitatively determined with a high resolution.

An advantage of the invention is that the force gradient of the optically-induced forces is relatively steep so that the cited transition between equilibriums is sudden and hence easy to precisely determine.

According to the invention, this procedure can be automated and used to calibrate the laser beam. The measurements can be repeated as often as desired, executed in a few seconds, and can also use the same particle in the environment in which it will later be used. In addition to absolute values of the optically-induced forces, relative values can also be measured such as symmetrical deviations of the optical beam and its intensity profiles both close and in the focal

range.

The above explanations on determining optically-induced forces are correspondingly applicable for determining bond strength since this can be described as an additional contribution to the electrical field force in the electrical capture area.

The invention can be used with any type of particle such as synthetic or biological cells or their components. The particle size can include the entire range of sizes that can be manipulated with laser tweezers, but preferably less than 200 μm .

Preferred exemplary embodiments of the invention will be further discussed in the following with reference to the accompanying drawings. The drawings show in:

Fig. 1: a schematic representation of the arrangement of field cage electrodes and an optical cage according to a first embodiment of the invention;

Fig. 2: another schematic representation of the arrangement of the field cage electrodes and an optical cage;

Fig. 3: a curve to illustrate experimental results;

Fig. 4: an example of the field distributions of an octopole field cage;

Fig. 5: a curve to illustrate the capture force of the field cage in the z direction shown in Fig. 4;

Fig. 6: a diagram of the arrangement of cells in an optical cage and a capture area according to a second embodiment of the invention;

Fig. 7: a diagram of the arrangement of cells in a micro-electrode arrangement according to a third embodiment of the invention;

Fig. 8: a diagram of the arrangement of cells in a micro-electrode arrangement according to a fourth embodiment of the invention; and

Fig. 9: a schematic representation of a device according to the invention.

The invention will be described in the following referring to the combination of an octopole field cage to form the capture area and an individual capture laser to form the optical cage. The invention can however be realized with any field cage shape or several laser beams.

Fig. 1 schematically illustrates an enlarged section of a microsystem according to the invention. The diagram only shows a microelectrode arrangement consisting of the microelectrodes 11 - 18 (without control lines) and a microscopic particle 113 between the microelectrodes in a suspension liquid. The microelectrodes are flat on opposing walls of the microsystem structure, and e.g. the x-y plane coincides with a substrate plane. The microelectrodes 11 - 18 are set up to be supplied with electrical potentials to form field gradients with one minimum field level. The technology of the electrode control to generate a specific minimum level is known per se and will therefore not be described in detail. The position of the minimum field level depends on the phases and amplitudes of the control potential at microelectrodes 11 - 18 and can be adjusted in a predetermined manner. The capture area or the electrical field cage is also termed a high-frequency cage since the microelectrodes are preferably supplied with high-frequency control potentials (see below for the frequency range) to manipulate the microscopic particles based on negative

dielectrophoresis.

In Fig. 1, the electrical high-frequency field cage created by the microelectrodes 11 - 18 is combined with the focused light beam 19 so that the focus 110a is in the electrical field of the microelectrodes, i.e., either in the field cage area 111 or directly adjacent 112 to the cage, while the minimum field level of the capture point 110b of the high frequency field cage is located at a distance (e.g. a fraction up to several particle diameters at position 114, identified by coordinates x_1 , y_1 , z_1).

The optically-induced field strengths acting on the particle 113 are measured according to the invention by first capturing the particle 113 in the focus of the laser beam and moving it by shifting the focus into the designated position (e.g. 114). The focus is shifted by mechanically changing the relative position of the microsystem and the source of the laser beam 19 by shifting devices and/or deflection devices for the laser beam which are known as such. By increasing the amplitude of the high-frequency signals applied to the electrodes, the electrical polarisation force of the field cage is increased until the particle 113 is torn from the laser focus and moved into the capture point 110b (transitional movement between the local equilibriums in the minimum field levels).

The particle can alternately be moved between the local equilibriums by increasing the laser power and moving the particle from the capture area of the field cage into the focus and/or by moving the locations of the capture point or focus to determine the path or distance of the minimum field level at which the transitional movement occurs. Since the electrical polarisation forces acting on the particle and the field distribution between the electrodes 11 - 18 are known, a direct proportionality exists between the measurable laser power in the focal range, the ampli-

tude of the electrical signals, and the optically-induced forces acting on the particle. By repeating the procedure and moving the particle 113 in any spatial direction, the optically-induced forces acting on the concrete particle can be quantitatively determined. This represents an electrical calibration of the optically-induced counter-forces that is easy to set up, and it allows forces in the range of fN up to a few hundred pN to be measured.

The optically-induced forces are hence determined in at least one case from the field or location properties of the particle 113 when it moves. The electrical polarisation forces acting on the particle 113 are calculated from the readily calculable field distribution between the micro-electrodes 11 - 18 and the given locations of the focus 110a and capture point 110b when the particle is moved. These locations can be measured with a viewing microscope. In all other instances, the optically-induced forces can be relatively measured based on the cited proportionality.

Fig. 2 shows an expanded diagram of a system to measure the optically-induced forces that act on a particle trapped in the focus. The microfield cage is formed by microelectrodes that are on facing surfaces of the substrate 27, 29. The substrates 27, 29 are separated by a spacer 28 that forms a suspension gap in which the investigated particle is exposed to the field of the microfield cage. The top substrate in Fig. 2 is sufficiently thin for the focus of the optical cage to be adjustable in the suspension area.

Through an opening 21, cells or other microparticles suspended in a solution are injected into the channel 22 and then reach the field cage 23 whose output electrodes 24a-d are subjected to a high-frequency field (kHz or MHz with any signal shape such as square, sinusoidal, triangular or any other signal shape, and with an amplitude of a few mV to some 10 V). By first applying electrical poten-

tial to the microelectrodes on one side, only an electrical field barrier for inserted microparticles is formed in the channel 22. If a particle is in the cage 23, the input electrodes 25a-d are also switched on and/or the flow is stopped. The particle is moved in the field cage area with the laser beam 26 as described above, and the optically-induced forces are measured. The phase shift typical for the electrode signals for electrical trapping to control two possible types of alternating fields and two rotation fields (2* AC field or 2*rot field) are summarised in Table 1.

Field type	El. 25b	El. 24b	El. 24d	El. 25d	El. 25a	El. 24b	El. 24c	El. 25c
AC field	0°	180°	0°	180°	0°	180°	0°	180°
AC field	0°	180°	0°	180°	180°	0°	180°	0°
Rot field	0°	90°	180°	270°	180°	270°	0°	90°
Rot field	0°	90°	180°	270°	90°	180°	270°	0°

table 1 Phase controls of the electrode signals of an octopole

In contrast to the AC field, torque is exerted on the particle in the rot field that causes the particle to rotate (last line of the table 1) which can also be used to determine force. The torque is compensated with the values of the next-to-last line of table 1.

In the present example, flat electrodes have been created on two glass substrates 27, 29 using methods known from semiconductor technology that are mounted liquid-tight from above by a spacer 28 so that they are immersed in the channel liquid 22. For the laser to be highly focussed, one of the glass substrates (27 in this instance) must be very

thin. In the present example, substrate 27 is 150 to 200 micrometers thick, and substrate 29 consists of 0.5 to 1 mm-thick glass or plastic.

As the laser output increases, so do the optically-induced forces so that correspondingly rising electrical field strengths, i.e., signal amplitudes, are necessary to bring about the cited transition between the minimum field levels. This is shown in Fig. 3 with reference to the experimental results. This curve shows the relationship between the laser output, i.e. the level of optically-induced force, and the amplitudes of the cage voltages, i.e. the amplitudes of the electrical potentials that are applied to the microelectrodes. As the laser output increases, greater amplitudes of the cage voltage are necessary to cause the particle to move between the focus and the capture point. It can clearly be seen that the capturing power of the optical trap is weaker in the z direction (top curve) than in the z or y direction (bottom curve).

The capture forces of the laser tweezers in the z direction shown in Fig. 5 result from the field distribution in Fig. 4 (obtained from a single numerical calculation taking into account the actual electrode dimensions in Fig. 1 and distances) and the passive electrical properties of the test particle (in this case, a latex bead with a diameter of eight micrometers). The curve shown refers to a signal amplitude of the cage electrodes of 10 V. Experimentally, amplitudes of up to 30 V were applied. Given a quadratic dependency on the capture force of the electrical cage, this corresponds to a maximum detectable force of approximately 150 pN with a resolution in the pN range and below. These measuring results correspond well with the theory of laser tweezers (see A. Ashkin in Biophys. J., Vol. 61, 1992, p. 569). Electrodes with a finer design allow much higher signal amplitudes and hence much higher electrically-induced forces to be generated so that substantially

greater force can be detected.

A particular advantage of the invention is that the method is quick and easy to carry out, especially in the environmental medium that will be later utilized using comparable conditions with the same particles for later investigation or manipulation. In addition, this procedure is not limited to specific particles and surface shapes; particles with any shape can be used. The force exerted on the adhering particle groups (such as aggregates, etc.) of any shape can also be measured.

Examples of measuring the force acting on adhering particle groups and exerting force on particles to manipulate particle groups will be described in the following with reference to Fig. 6 - 8.

Fig. 6 shows a microelectrode arrangement in an octopole shape with microelectrodes 61 - 68 analogous to Fig. 1. The microelectrodes 61 - 64 and 65 - 68 are in a microsystem set up for a process according to the invention in two spaced levels to form an electrical field cage with a minimum field level that forms the capture area or point. The electrical field cage is in the microelectrode arrangement that is sketched by the cube in Fig. 6. Reference number 69 refers to a light beam (preferably laser beam) focussed in the microelectrode arrangement. A first particle as a biological cell 611 is in the focus of the light beam 69. A second particle that is also a biological cell 612 in the illustrated example is in the capture point of the electrical field cage. The bond force between the particles can be determined as discussed below analogous to measuring the optically-induced force by observing the transitional movement of a particle from the focus to the capture area as discussed above.

First two cells 611, 612 are sequentially introduced into

the microelectrode arrangement 61 - 81. This is preferably done with the injection process explained with reference to Fig. 2 where the microelectrode arrangement is connected to a channel structure of a fluid microsystem. The first cell 611 is injected into the microelectrode arrangement and held in the electrical capture area (trap area) after the octopole field is completely under control. Then the first cell 611 is taken by the optical cage formed by the laser beam 69 and positioned at a distance from the capture area. Then the second cell 612 is injected and positioned in the trap area, e.g. in the middle of the microelectrode arrangement 61 - 68. However, any other technique can be used to introduce the microscopic particles into the microelectrode arrangement. Depending on the use, both particles can be like or different biological cells or biological cells, cell components, and/or synthetic particles with specific active ingredients.

Subsequently, the cells 611, 612 contact each other, and an adhesive bond is formed between both cells. The adhesive bond can e.g. arise from one of the following techniques. First off, it is possible to release the first cell 611 by shutting off the laser beam 69 and then move it under the effect of the electrical field to the capture area of the electrical field cage where it contacts the second cell 612, and the adhesive bond is formed. It is also possible to adjust the focus of the laser beam 69 in reference to the capture area so that the first cell 611 contacts the second cell 612 or is even pressed against it with a predetermined force. The mutual forces of the cells pressed against each other can be derived from the electrical field strength in the capture area and the optical force in the focus of the laser beam 69 e.g. determined by the above-cited technique. To quantitatively compare the measured bonding force, the adhesive bond is set for a predetermined period (e.g. approx. 0.1 - 10 sec.). Longer times up to e.g. 1,000 seconds are also possible.

After the contact period that is set depending on the application, the binding forces (interactive forces, adhesion strength) between the cells can be determined as follows. The force is measured analogously to the above-cited measurement of the optically-induced force acting on individual particles by varying the field properties and determining the field strengths in the electrical capture area and optical cage at which the cells tear away from each other. For example, at a given electrical potential amplitude, the laser beam 69 focussed on the first cell 611 is moved repeatedly so that the focus moves away from the capture area. If the first cell 611 does not move with the focus, the focus is reset and the cell is subjected to stepped-up light power. When the optical force induced by the light power is known, the force it takes to tear two cells apart and hence the mutual binding force can be calculated from the light power it takes to entrain the first cell 611 by moving the focus. Other possibilities for measuring this force are to vary stepwise the electrical potential amplitudes and move the location of the capture area by correspondingly controlling the microelectrodes 61 - 68. Combinations of both techniques can be used to vary the field properties.

A particular advantage of this procedure is that it can be repeated with a given pair of particles, and any desired cell contact time can be precisely set.

Preferred applications for the procedure described with reference to Fig. 6 (that until now was unacceptably expensive) are to contact two or more like or different biological cells for a precise period. The process is therefore useful for pharmacological and medical diagnostic tests. An example of a process in this context is to stimulate the second cell 612 by temporarily contacting it with the first cell 611 to change the binding behaviour of

the second cell 612. The changed binding behaviour can then be tested by coupling a third cell (not shown). Another example is to screen peptide or other molecular libraries by introducing a first type of cell into the microelectrode arrangement. The surface receptors of the first cell are then contacted with a test or active substance from a molecular library through the suspension solution in the microsystem. Then a second cell (or a synthetic particle with surface molecules that bond well) is brought to the first cell. The above-explained measurement of the bonding force can yield one of the following results. If the bonding sites of the first cell are already saturated with the active substance, there is no (or only a weak) binding to the test cell. Otherwise, a strong bond is formed with the test cell. Biological cells can be accordingly evaluated and sorted in reference to their reaction of specific active substances.

Another example of stimulating biological cells will be explained below with reference to Fig. 7. Fig. 7, like Fig. 1 and 6, shows a microelectrode arrangement with microelectrodes 71 - 78 and a light beam 79 focussed in the microelectrode arrangement.

Like the above-explained injection or flow principle, a first cell 711 is captured in the electrical capture area of the microelectrode arrangement 71 - 78 and positioned. A second cell 712 or a synthetic particle with an active ingredient is captured with the light beam 79 and moved along a set path 713 past the first cell 711, or it contacts the first cell for a predetermined time. Even when the cells or particles do not bond or adhere, a chemical signal can be transmitted when the surfaces contact each other that can result in a subsequently analysable change in one or both cells. Instead of the individual cells, cell groups or aggregates can be joined and separated to mutually stimulate each other for a set period under the effect

of predetermined forces.

This procedure to stimulate biological cells or cell groups has numerous applications in medicine and biotechnology. Stimulation e.g. by applying a medication from the environmental solution that up to now could only be done with a great deal of effort can now be carried out for specific cells under defined and reproducible conditions. In living organisms, such stimulations (or inhibitions) usually occur from interactions between cells that are closely adjacent or must contact each other. The conditions that arise in organisms can hence be advantageously simulated with the process according to the invention.

The procedure illustrated by Fig. 7 can advantageously be with or without the above-cited measurement of optically-induced forces by setting predetermined field properties. The same holds true for the process explained with reference to Fig. 8 for exerting set forces on cells or cell groups to form aggregates.

Fig. 8 shows a microelectrode arrangement with microelectrodes 81 - 88 and a light beam 89 in the microelectrode arrangement.

According to the above-discussed injection or flow technique, numerous biological cells are injected into the interior of the microelectrode arrangement. With the optical cage of the light beam 89, cells are specifically moved into the capture area and positioned there, possibly relative to existing cells. For example, four cells 811 - 814 are in the electrical capture area in Fig. 8 to which a fifth cell 815 is added at a set position (corresponding to the arrow). The fifth cell 815 is pressed for a set period with a set force against the formed cell group 811 - 814 to allow the formation of bonding forces in this predetermined relative position.

Any cell aggregate with a predetermined shape can be constructed in this manner. This procedure can also be implemented using negatively polarised synthetic microparticles that can be repulsed by the microelectrodes in the electrical capture area (negative dielectrophoresis).

A device according to the invention consists of an arrangement of a fluid microsystem 91, an illumination device 92 to generate an optical cage in a microelectrode arrangement of the microsystem 91, where the microsystem 91 and the illumination device 92 are adjustable relative to each other with an adjusting device 93, and a monitoring device and/or sensor 94 (such as a microscope) as schematically portrayed in Fig. 9. The microsystem is provided with a fluid and potential control 95 as known as such. The illumination device 92 is e.g. a prior-art pair of laser tweezers as known as such that can contain a diode laser or a semiconductor laser as the light source and has a microscope arrangement for focussing.

CLAIMS (as originally filed and published)

1. A procedure to measure or exert optically-induced forces on at least one particle in the focus of an optical cage with the following steps:
 - a) the focus is positioned in a microelectrode arrangement with a three-dimensional electrical field that has a field gradient which forms an electrical capture area and the focus is at a distance from the capture area, and
 - b) the amplitude of the electrical field, the light power of the light beam forming the optical cage, and/or the distance of the capture area from the focus are varied to detect at which of said varied field properties the particle is moved from the focus to the capture area or vice versa, or to at least to temporarily move the particle into the capture area.
2. The process according to claim 1 in which a particle is placed in the focus or capture area to measure optically-induced forces, and the optically-induced forces are measured from the amplitude of the electrical field and the distance of the capture area from the focus when the particle moves from the focus to the capture area or vice versa.
3. The process according to claim 2 in which the optically-induced forces are repeatedly measured for all relevant directions in space corresponding to mutual alignment of the positions of the focus to the capture area.

4. The process according to claim 2 or 3 in which the optical cage is calibrated by determining the relationship between the light power to generate the optical cage and the forces induced on a particle in the optical cage.
5. The process according to one of claims 2 - 4 in which the distance between the focus and capture area is at least one-tenth of the particle diameter.
6. The process according to one of the above claims in which the capture area is a capture point that is in the beam field of the optical cage so that the particle moves back and forth between the capture point and focus when the amplitude of the electrode signals or light power is lowered or increased, and the associated value of the amplitude is used to measure the optically-induced forces.
7. The process according to claim 1 in which at least one particle is in the focus and at least one second particle is in the capture area to determine bonding forces between the microscopic particles, whereby the first and second particles contact each other for a predetermined contact period, and then the amplitude of the electrical field, the light power and/or the distance of the capture area from the focus are varied until the first particle can be moved with the focus from the capture area and second particle, whereby the binding forces between the particles are determined from the amplitude of the electrical field and the light power when the particle moves.
8. The process according to one of the above claims in which the electrodes of the microelectrode arrangement are alternately supplied with signals phase-shifted 180° and/or with rotation-generating signals with a

predetermined phase division.

9. The process according to one of the above claims in which the capture area is separated by at least one field barrier from the optical cage.
10. The process according to claim 1 in which numerous particles are sequentially injected into the capture area that are positioned in set positions with the optical cage in the capture area relative to possibly existing particles in the capture area.
11. The process according to one of claims 1 - 6 in which the light beam of the optical cage is adjusted and/or the capture quality, symmetry or other calibration properties of the optical cage are measured.
12. The process according to one of claims 1 - 6 in which the particle is characterised based on the measured optically-induced forces.
13. The process according to one of the above claims in which the particle movement is optically and/or electrically detected.
14. The process according to one of the above claims in which the particles are synthetic or natural particles with a size below 200 μm .
15. The process according to one of the above claims in which the particles are biological cells or their components.
16. The process according to one of the above claims in which the transitional movement of the particle from the capture area to the focus or vice versa is used to adjust the optical cage.

17. A device to measure or exert optically-induced forces on at least one particle in the focus of an optical cage that comprises:
 - a fluid microsystem with a microelectrode arrangement that is set up to form a three-dimensional electrical field with an electrical capture area,
 - an illuminating device that is set up to form an optical cage in the microelectrode arrangement of the microsystem, and
 - an monitoring and/or detection device to measure the movement of particles in the microelectrode arrangement.
18. The device according to claim 17 in which the microelectrode arrangement comprises flat electrodes that are in groups on two spaced substrates of which at least one is transparent.
19. The device according to claim 18 in which the thickness of the transparent substrate is less than 500 μm .
20. The device according to claim 18 in which the electrodes are attached to facing surfaces of the substrates, and the substrates are separated from each other by a spacer that forms a suspension area into which the focus of the optical cage can be coupled by the illumination device through one of the two substrates.
21. The device according to claim 20 in which the suspension area is part of a channel structure through which the particles are introduced by means of a flow of solution into the field of the microelectrode arrangement.
22. The device according to one of claims 17 - 21 in which

the microelectrode arrangement comprises numerous electrodes that are set up to generate a multipole field with an electrical field distribution symmetrical in the x, y and/or z direction.

23. The device according to one of claims 17 - 22 in which the electrodes are coated with an insulating, dielectric layer or consist of metals that are essentially inert to the suspension liquid in the microsystem.
24. The device according to claim 23 in which the electrodes consist of platinum, titanium, tantalum or gold.
25. The device according to one of claims 17 - 24 in which electrodes are constructed in three-dimensional shapes using methods from semiconductor technology, or are constructed using hybrid techniques.
26. The use of a procedure or a device according to one of the prior claims to calibrate a laser tweezer.
27. The use of a procedure or a device according to one of the prior claims to selectively stimulate biological cells.

ABSTRACT

To measure or exert optically-induced forces on at least one particle in the focus of an optical cage, the following steps are taken:

- a) the focus is positioned in a microelectrode arrangement with a three-dimensional electrical field that has a field gradient which forms an electrical capture area, and the focus is at a distance from the capture area, and
- b) the amplitude of the electrical field, the light power of the light beam forming the optical cage, and/or the distance of the capture area from the focus are varied to detect which varied field property moves the particle from the focus to the capture area or vice versa, or at least to temporarily move the particle into the capture area.

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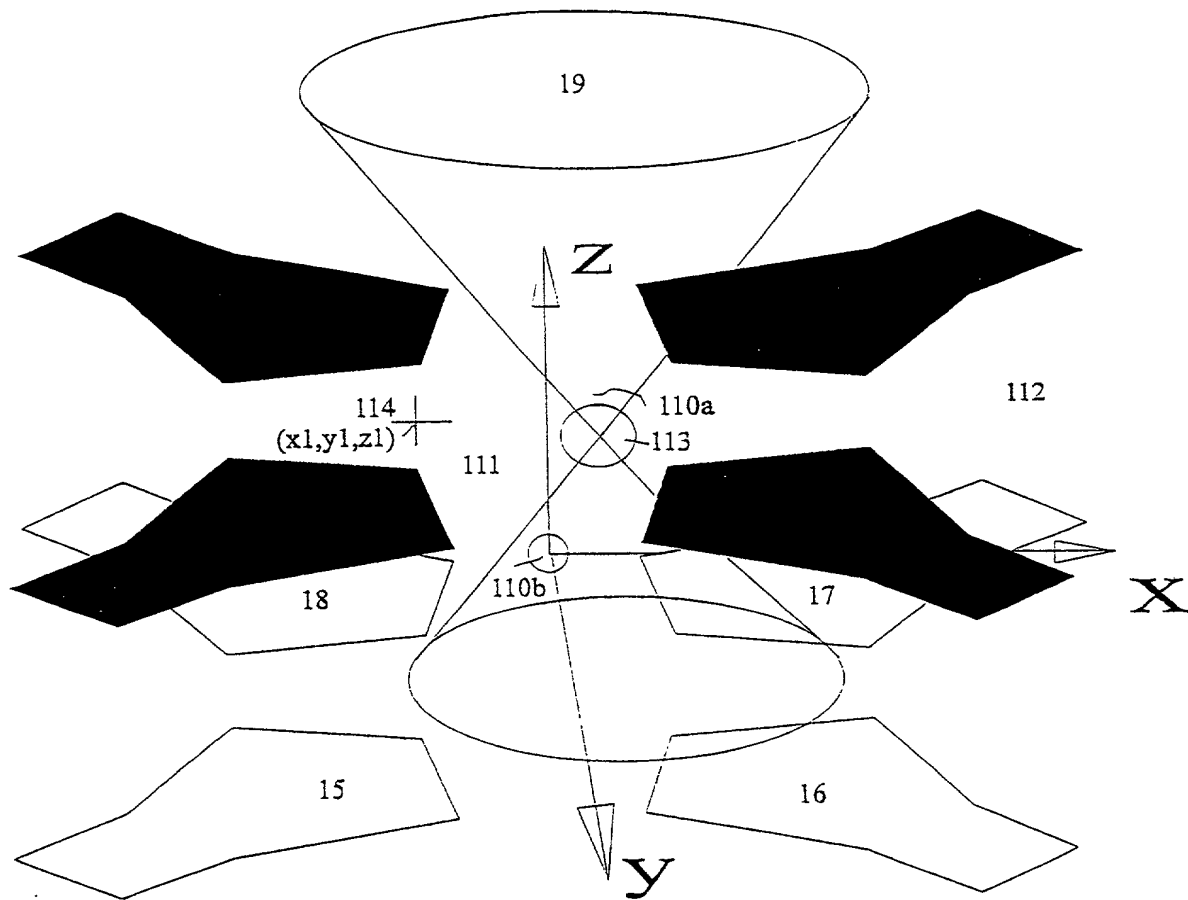


Fig. 1

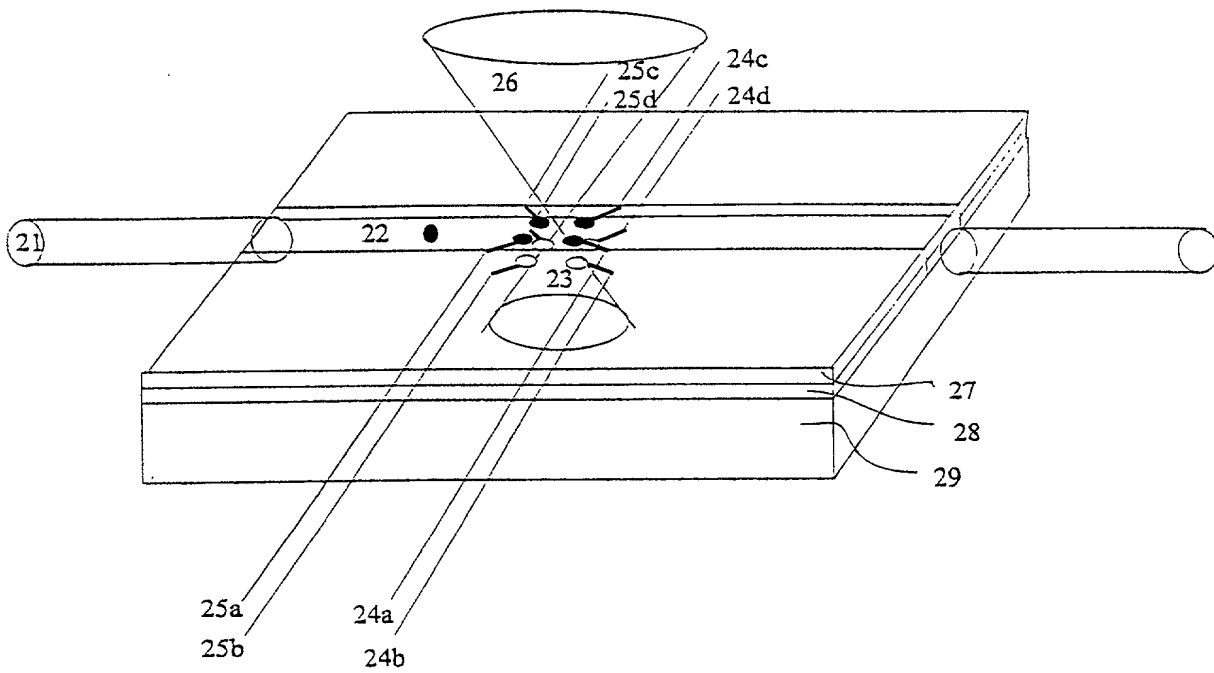


Fig. 2

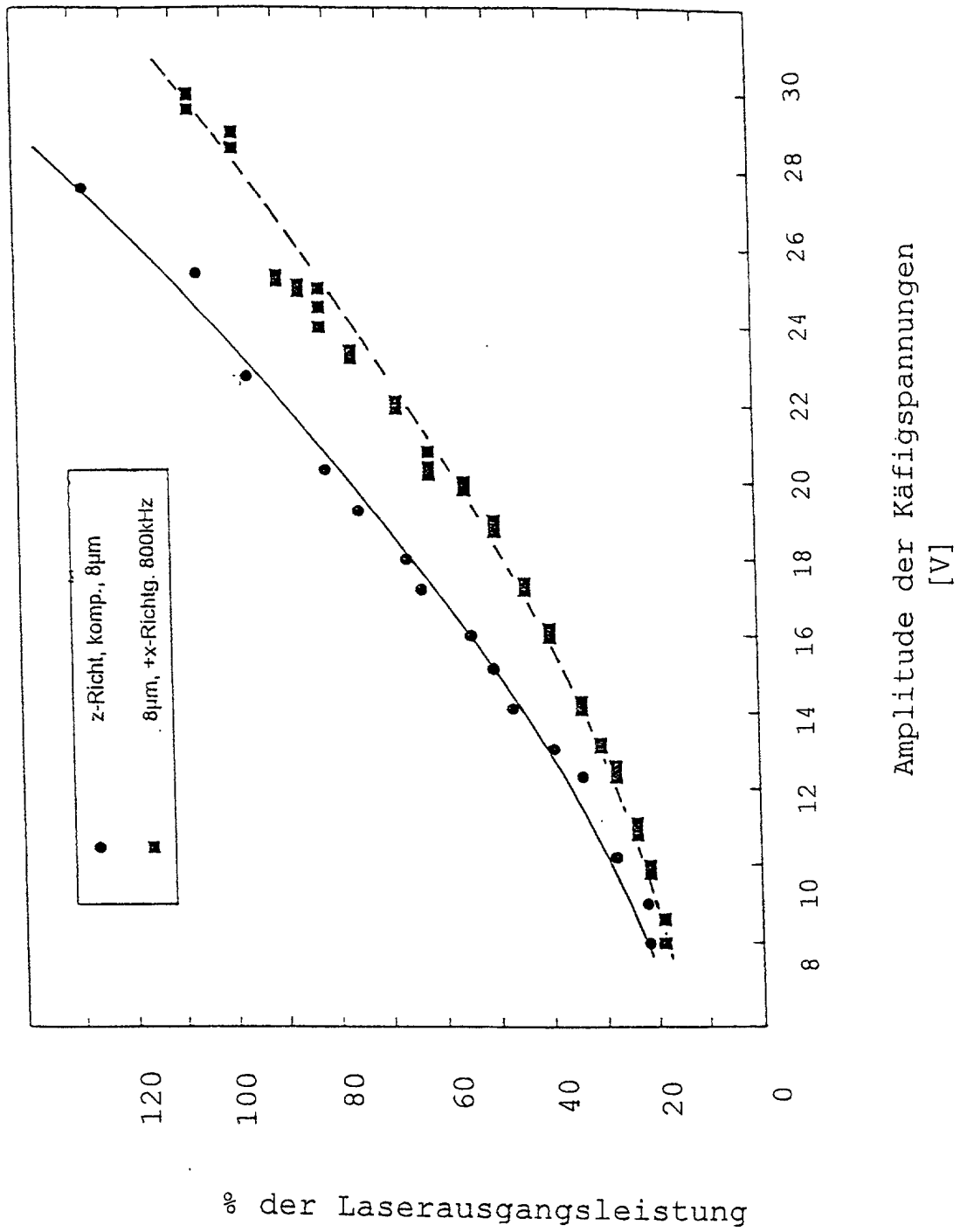


Fig. 3

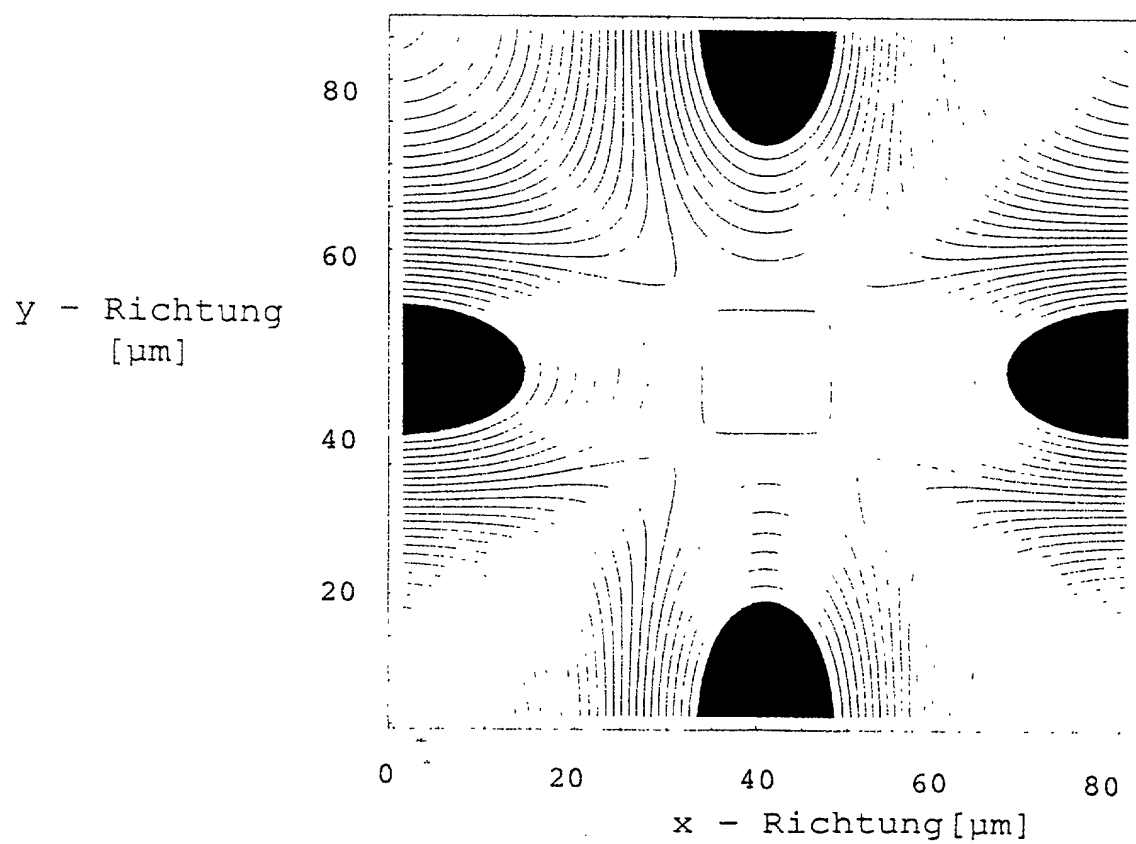
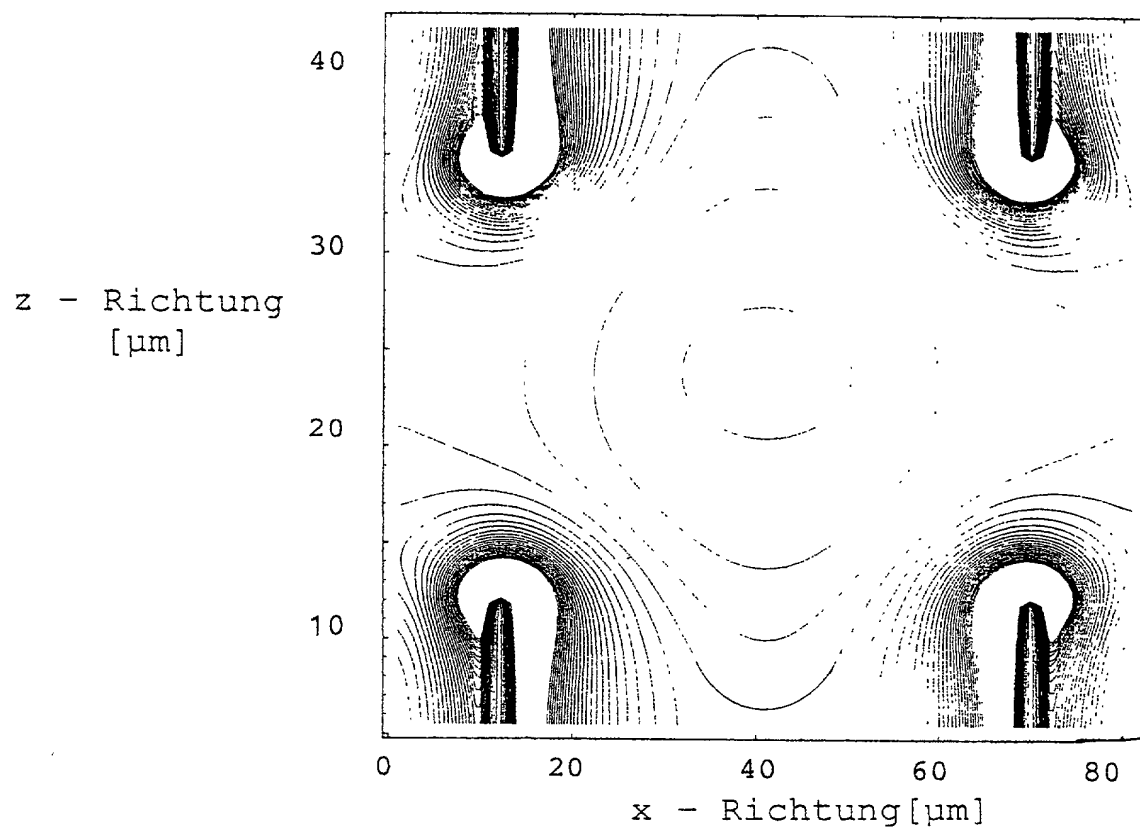
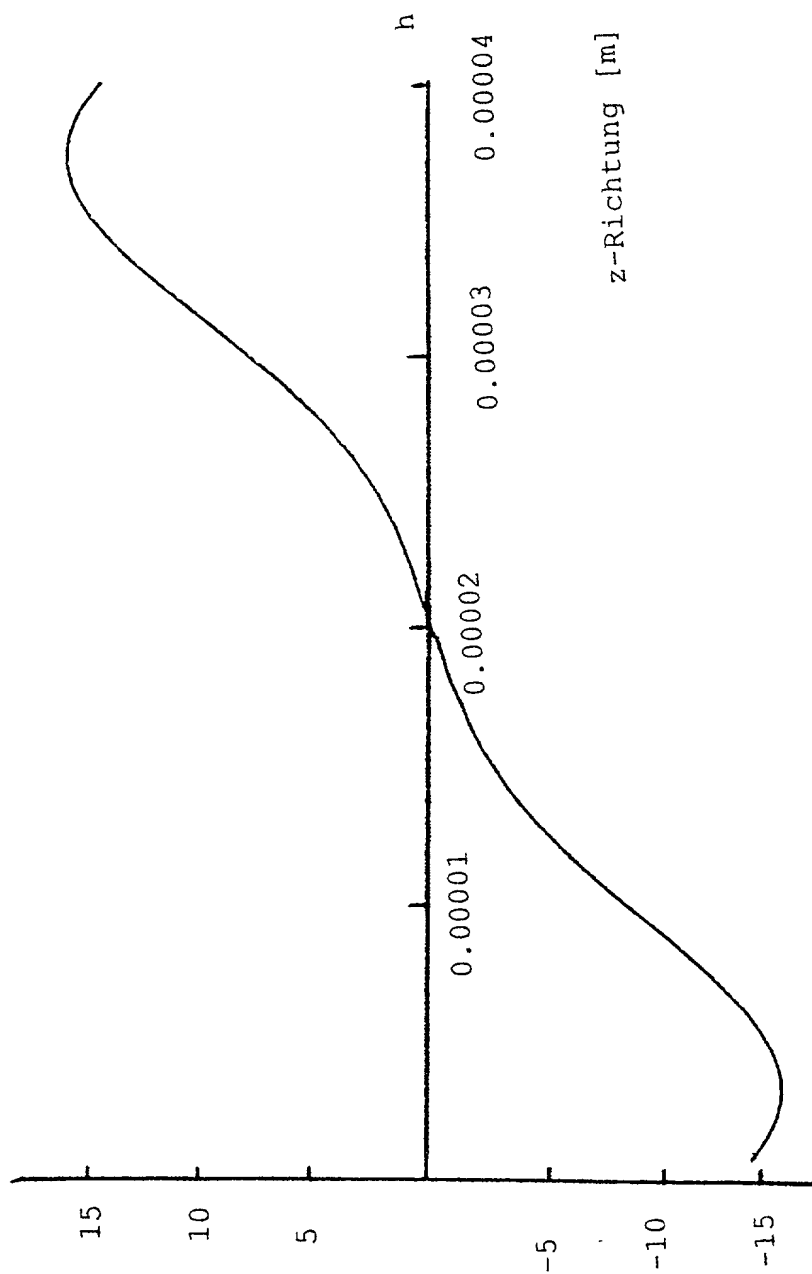


Fig. 5

Fangkräfte F_q [pN]



z-Richtung [m]

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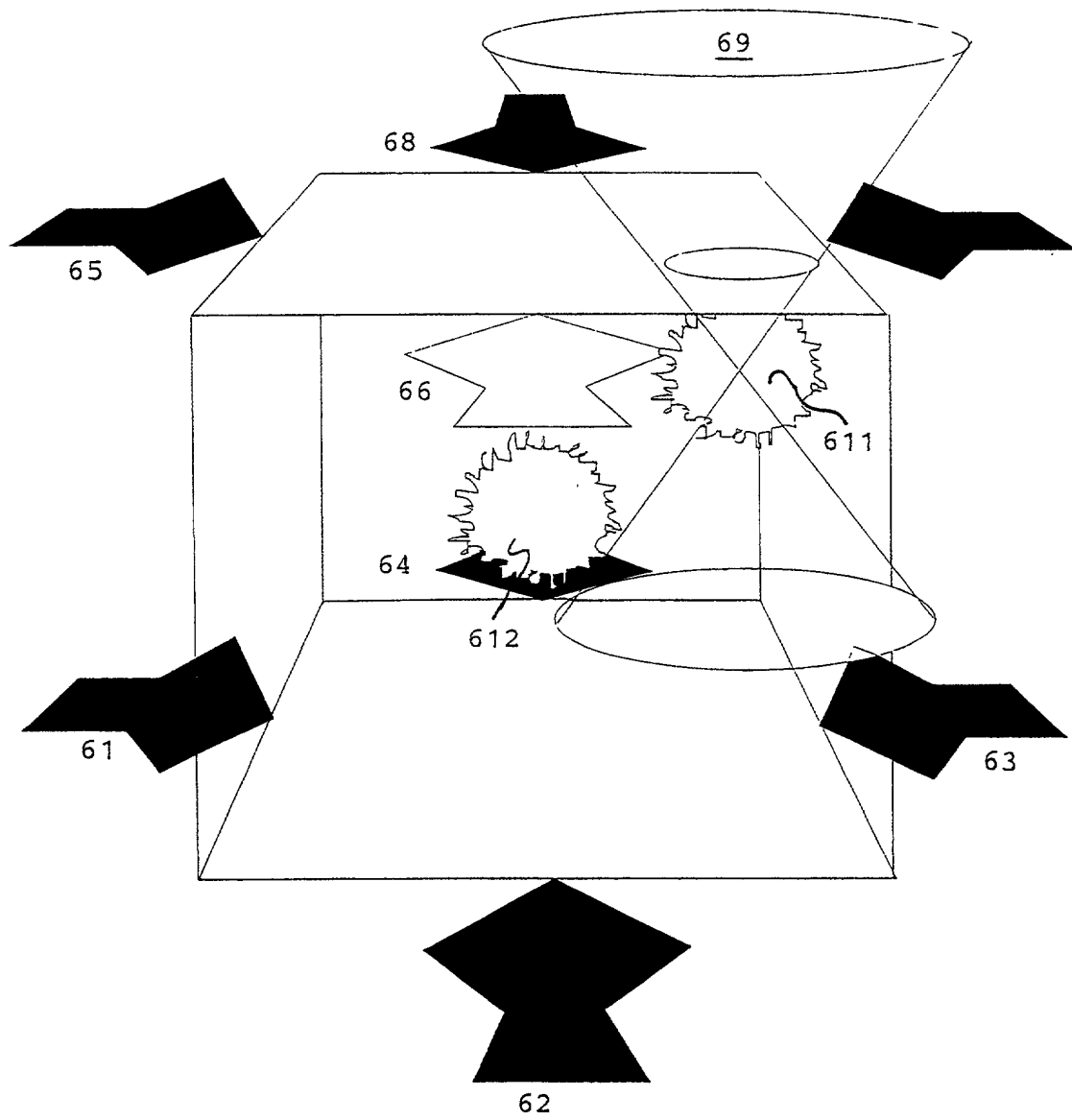


Fig. 6

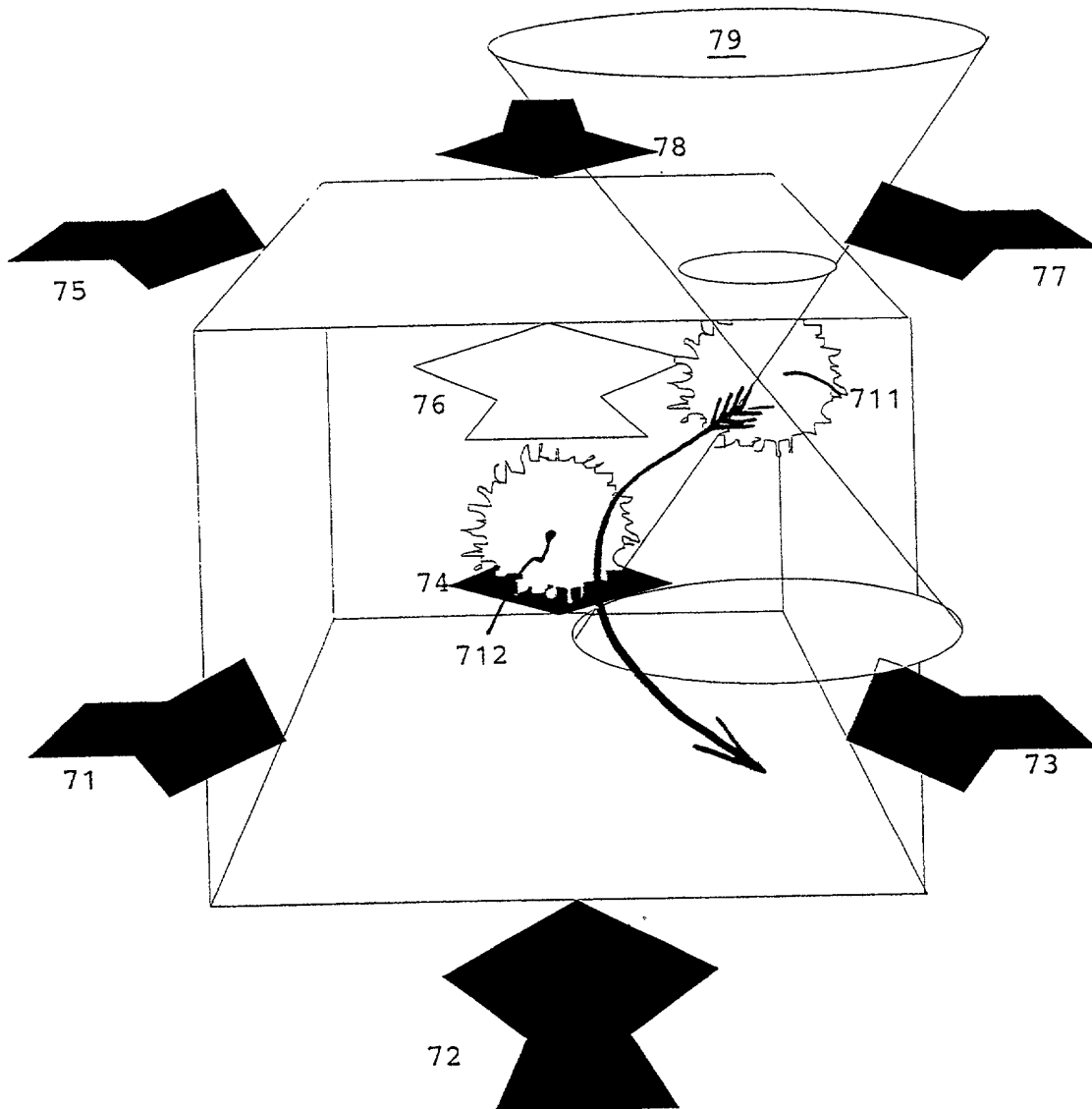


Fig. 7

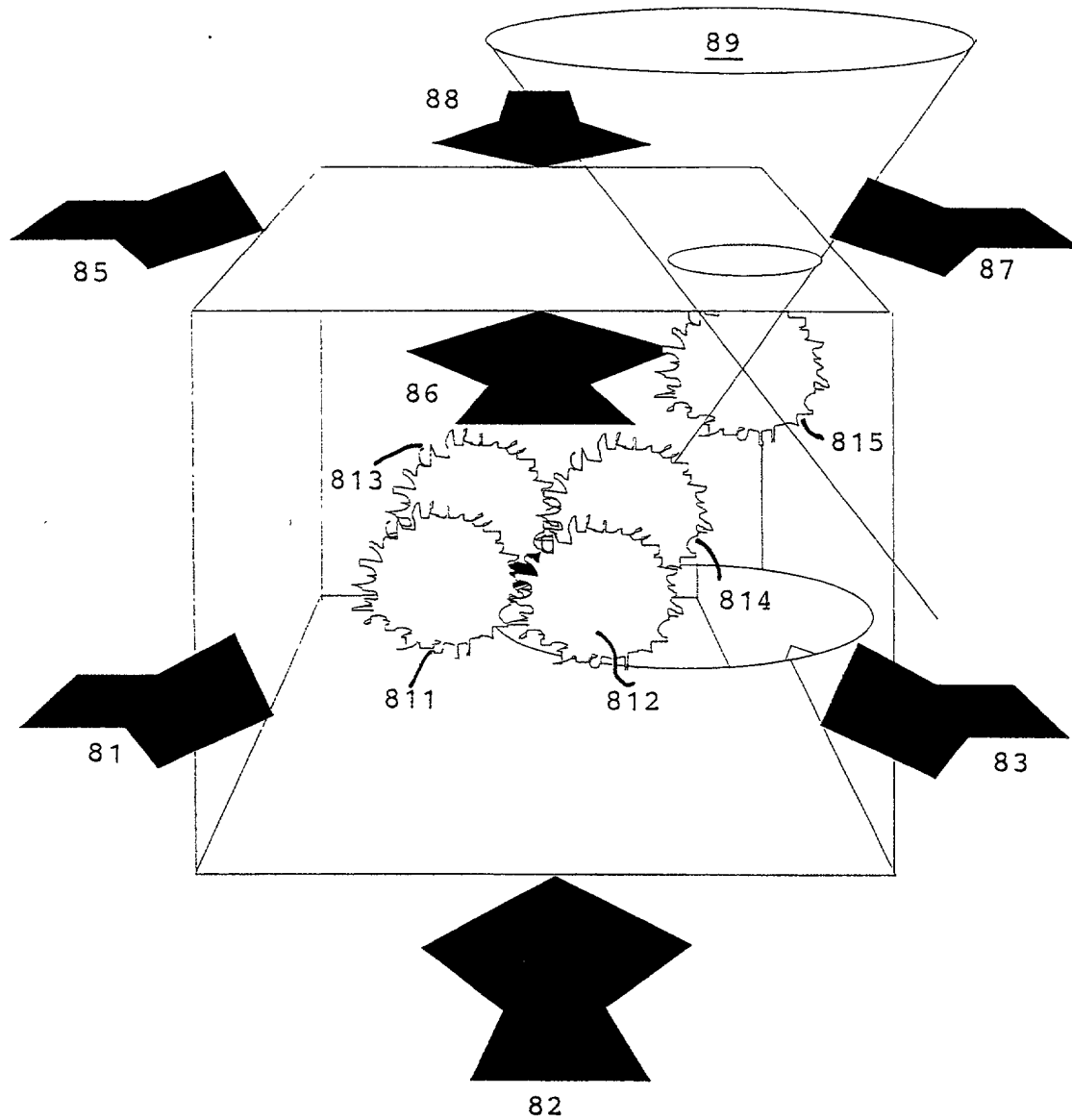


Fig. 8

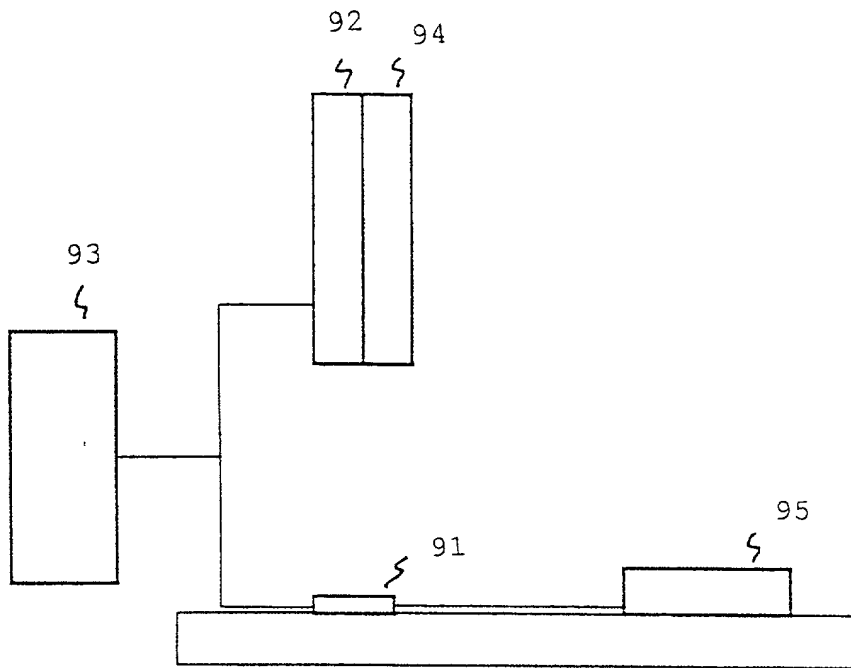


Fig. 9

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**COMBINED DECLARATION
AND POWER OF ATTORNEY****(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND USE LASER WEEZERS

This declaration is of the following type:

- ☐ original
- ☐ design
- ☒ national stage of PCT.
- ☐ divisional
- ☐ continuation
- ☐ continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*

- (a) ☐ is attached hereto.
- (b) ☐ was filed on as Application Serial No. and was amended on *(if applicable)*
- (c) ☒ was described and claimed in PCT International Application No. PCT/EP98/08370 filed December 21, 1998 on and was amended on *(if applicable)*.

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56

☐ In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

- (d) ☐ no such applications have been filed
- (e) ☒ such applications have been filed as follows:

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PRIOR FOREIGN PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
COUNTRY	APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
			PRIORITY CLAIMED UNDER 35 USC 119
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ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
Germany	197 57 785 7	28-12-97	<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)

Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836; Henry Tang, Reg. No. 29,705; Robert C. Scheinfeld, Reg. No. 31,300; John A. Fogarty, Jr., Reg. No. 22,348; Louis S. Sorrell, Reg. No. 32,139; Rochelle K. Seide, Reg. No. 32,300; Gary M. Bunker, Reg. No. 33,841; Marta E. Delsignore, Reg. No. 32,689; and Lisa B. Kole, Reg. No. 35,225 of the firm of BAKER BOTTS L.L.P. with offices at 30 Rockefeller Plaza, New York, New York 10112 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

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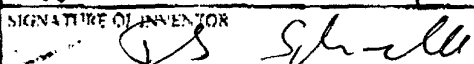

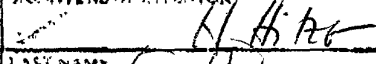
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that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME <u>1-00</u> <u>Fuhr</u>	FIRST NAME <u>Günter</u>	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY <u>Berlin</u> DEX	STATE or FOREIGN COUNTRY <u>GERMANY</u>	COUNTRY OF CITIZENSHIP <u>GERMANY</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Kavalierstraße 15</u>	CITY <u>Berlin</u>	STATE or COUNTRY <u>GERMANY</u> ZIP CODE <u>D-13187</u>
DATE <u>07/11/2000</u>	SIGNATURE OF INVENTOR 		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME <u>2-00</u> <u>Schnelle</u>	FIRST NAME <u>Thomas</u>	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY <u>Berlin</u> DEX	STATE or FOREIGN COUNTRY <u>GERMANY</u>	COUNTRY OF CITIZENSHIP <u>GERMANY</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Koppenstraße 65</u>	CITY <u>Berlin</u>	STATE or COUNTRY <u>GERMANY</u> ZIP CODE <u>D-10243</u>
DATE <u>07/11/2000</u>	SIGNATURE OF INVENTOR 		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME <u>3-00</u> <u>Müller</u>	FIRST NAME <u>Torsten</u>	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY <u>Berlin</u> DEX	STATE or FOREIGN COUNTRY <u>GERMANY</u>	COUNTRY OF CITIZENSHIP <u>GERMANY</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Hartneggstraße 100</u>	CITY <u>Berlin</u>	STATE or COUNTRY <u>GERMANY</u> ZIP CODE <u>D-12439</u>
DATE <u>07/12/2000</u>	SIGNATURE OF INVENTOR 		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME <u>4-00</u> <u>Hitzler</u>	FIRST NAME <u>Hermine</u>	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY <u>Berlin</u> DEX	STATE or FOREIGN COUNTRY <u>GERMANY</u>	COUNTRY OF CITIZENSHIP <u>GERMANY</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Reilerstraße 6</u>	CITY <u>Berlin</u>	STATE or COUNTRY <u>GERMANY</u> ZIP CODE <u>D-12681</u>
DATE <u>07/12/2000</u>	SIGNATURE OF INVENTOR 		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME <u>5-00</u> <u>Greulich</u>	FIRST NAME <u>Karl-Otto</u>	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY <u>Heidelberg</u> DEX	STATE or FOREIGN COUNTRY <u>GERMANY</u>	COUNTRY OF CITIZENSHIP <u>GERMANY</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Plöck 27</u>	CITY <u>Heidelberg</u>	STATE or COUNTRY <u>GERMANY</u> ZIP CODE <u>D-69117</u>
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	LAST NAME <u>Monajembashi</u>	FIRST NAME <u>Shamou</u>	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY <u>Heidelberg</u>	STATE or FOREIGN COUNTRY <u>GERMANY</u>	COUNTRY OF CITIZENSHIP <u>GERMANY</u>
POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Fritz-Frey-Straße 2</u>	CITY <u>Heidelberg</u>	STATE or COUNTRY <u>GERMANY</u> ZIP CODE <u>D-69121</u>
DATE	SIGNATURE OF INVENTOR		

07.07.2000 18:28 212

FROM BAKERBOTTTS

(FRI) 7. 7'00 15:30/ST. 13:28 NO. 4861225557 F 6

BAKERBOTTTS I.P.

FILE NO.: A33331 PCT USA

FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY ZIP CODE
DATE	SIGNATURE OF INVENTOR		

Check proper box(es) for any added page(s) forming a part of this declaration

- ☐ Signature for ninth and subsequent joint inventors Number of pages added _____
- ☐ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor
Number of pages added _____
- ☐ Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47.
Number of pages added _____

FROM BAKERBOTTIS

(FRI) 7. 7:00 13:29/ST. 13:28/NO. 4860225357 P 3

BAKER BOTIS LLP

FILE NO.: A33331 PCT USA

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND USE LASER TWEEZERS

This declaration is of the following type:

- ☐ original
- ☐ design
- ☒ national stage of PCT.
- ☐ divisional
- ☐ continuation
- ☐ continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*

(a) ☐ is attached hereto

(b) ☐ was filed on as Application Serial No. and was amended on *(if applicable)*.

(c) ☒ was described and claimed in PCT International Application No. PCT/EP98/08370 filed December 21, 1998 on and was amended on *(if applicable)*.

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56

☐ In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

(d) ☐ no such applications have been filed.

(e) ☒ such applications have been filed as follows:

FROM BAKERBOTT

(FRI) 7. 7'00 13:30/ST. 13:28/NO. 4860225357 P 4

BAKER BOTTS LLP

FILE NO.: A33331 PCT USA

PRIOR FOREIGN PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
COUNTRY	APPLICATION NO	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
			PRIORITY CLAIMED UNDER 35 USC 119 <input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
Germany	197 57 785.7	28-12-97	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836; Henry Tang, Reg. No. 29,705; Robert C. Schenfeld, Reg. No. 31,300; John A. Fogarty, Jr., Reg. No. 22,348; Louis S. Sorell, Reg. No. 32,439; Rochelle K. Seide, Reg. No. 32,300; Gary M. Butter, Reg. No. 33,841; Marta E. Delsignore, Reg. No. 32,689; and Lisa B. Kole, Reg. No. 35,225 of the firm of BAKER BOTTS L.L.P., with offices at 30 Rockefeller Plaza, New York, New York 10112, as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO: BAKER BOTTS L.L.P. 30 ROCKEFELLER PLAZA, NEW YORK, N.Y. 10112 CUSTOMER NUMBER: 21003	DIRECT TELEPHONE CALLS TO: BAKER BOTTS L.L.P. (212) 705-5000
---	--

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge

FROM BAKERBOTTIS

(FRI) 7. 7'00 13:30/ST. 13:28/NO. 4960225357 P 5
BAKERBOTTIS LLP.

FILE NO.: A33331 PCT USA

- that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME Fuhr	FIRST NAME Günter	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Kavalierstraße 15	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME Schnelle	FIRST NAME Thomas	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Koppenstraße 65	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME Müller	FIRST NAME Torsten	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Hartriegelstraße 100	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME Hitzler	FIRST NAME Hermine	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Reilerstraße 6	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME Greulich	FIRST NAME Karl-Otto	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Heidelberg	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Plöck 27	CITY Heidelberg	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	LAST NAME Monajembashi	FIRST NAME Shamsh	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Heidelberg	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Fritz-Frey-Straße 2	CITY Heidelberg	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		

FROM BAKERBOTTTS

(FRI) 7. 7'00 13:30/ST. 13:28 NO. 4860225357 P 6

BAKER BOTTS I.T.P.

FILE NO.: A33331 PCT USA

FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY ZIP CODE
DATE	SIGNATURE OF INVENTOR		

Check proper box(es) for any added page(s) forming a part of this declaration

- ☐ Signature for ninth and subsequent joint inventors. Number of pages added _____
- ☐ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor.
Number of pages added _____
- ☐ Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47.
Number of pages added _____

FROM BAKERBOTTIS

(FRI) 7. 7' 00 13:29/ST. 13:28/NO. 4960225357 P 3
BAKER BOTIS LLP.

FILE NO.: A33331 PCT USA

**COMBINED DECLARATION
AND POWER OF ATTORNEY****(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESSES AND DEVICES TO MEASURE, CALIBRATE, AND USE LASER TWEEZERS

This declaration is of the following type:

- ☐ original
☐ design
☒ national stage of PCT.
☐ divisional
☐ continuation
☐ continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*(a) ☐ is attached hereto.(b) ☐ was filed on as Application Serial No. and was amended on *(if applicable)*.(c) ☒ was described and claimed in PCT International Application No. PCT/EP98/08370 filed December 21, 1998 on and was amended on *(if applicable)*.**Acknowledgement of Review of Papers and Duty of Candor**

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

☐ In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.**Priority Claim**

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))(d) ☐ no such applications have been filed.(e) ☒ such applications have been filed as follows:

FROM BAKERBOTT

(FRI) 7. 7' 00 13:30/ST. 13:28/NO. 4860225357 P 4

BAKER BOTTS LLP

FILE NO.: A33331 PCT USA

PRIOR FOREIGN PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
COUNTRY	APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
			PRIORITY CLAIMED UNDER 35 USC 119 <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
			<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
			<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
Germany	197 57 785.7	28-12-97	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
			<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>
			<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/>

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number(s)	Filing Date

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
--------------------------	---------------	---

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
--------------------------	---------------	---

Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836; Henry Tang, Reg. No. 29,705; Robert C. Schoenfeld, Reg. No. 31,300; John A. Fogarty, Jr., Reg. No. 22,348; Louis S. Sorell, Reg. No. 32,439; Rochelle K. Seide, Reg. No. 32,300; Gary M. Butter, Reg. No. 33,841; Marta E. Delsignore, Reg. No. 32,689; and Lisa B. Kole, Reg. No. 35,225 of the firm of BAKER BOTTS L.L.P., with offices at 30 Rockefeller Plaza, New York, New York 10112, as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO: BAKER BOTTS L.L.P. 30 ROCKEFELLER PLAZA, NEW YORK, N.Y. 10112 CUSTOMER NUMBER: 21003	DIRECT TELEPHONE CALLS TO: BAKER BOTTS L.L.P. (212) 705-5000
--	---

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge

FROM BAKERBOTT

(FRI) 7. 7'00 13:30/ST. 13:28. NO. 4660225357 F 5

BAKERBOTT'S LLP

FILE NO.: A33331 PCT USA

- that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME Fuhr	FIRST NAME Günter	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Kavalierstraße 15	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME Schnelle	FIRST NAME Thomas	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Koppenstraße 65	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME Müller	FIRST NAME Torsten	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Hartnigelstraße 100	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME Hitzler	FIRST NAME Hermine	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Berlin	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Reilerstraße 6	CITY Berlin	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME Greulich	FIRST NAME Karl-Otto	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Heidelberg	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Plöck 27	CITY Heidelberg	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	LAST NAME Monajembashi	FIRST NAME Shamir	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY Heidelberg	STATE or FOREIGN COUNTRY GERMANY	COUNTRY OF CITIZENSHIP GERMANY
POST OFFICE ADDRESS	POST OFFICE ADDRESS Pritz-Frey-Straße 2	CITY Heidelberg	STATE or COUNTRY GERMANY
DATE	SIGNATURE OF INVENTOR		
30.08.2000	Shamir Monajembashi		

FROM BAKERBOTTTS

(FRI) 7. 7' 00 13:30/ST. 13:28/NO. 4960225357 P 6

BAKER BOTTS I.L.P.

FILE NO.: A33331 PCT USA

FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY
DATE	SIGNATURE OF INVENTOR		

Check proper box(es) for any added page(s) forming a part of this declaration

- ☐ Signature for ninth and subsequent joint inventors. Number of pages added _____
- ☐ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor.
Number of pages added _____
- ☐ Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47.
Number of pages added _____